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REVIEW

Pharmacologic management of *Mycobacterium ulcerans* infection

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ABSTRACT

Introduction: Pharmacological treatment of Buruli ulcer (*Mycobacterium ulcerans* infection; BU) is highly effective, as shown in two randomized trials in Africa.

Areas covered: We review BU drug treatment – in vitro, in vivo and clinical trials (PubMed: '(Buruli OR (Mycobacterium AND ulcerans)) AND (treatment OR therapy).' We also highlight the pathogenesis of *M. ulcerans* infection that is dominated by mycolactone, a secreted exotoxin, that causes skin and soft tissue necrosis, and impaired immune response and tissue repair. Healing is slow, due to the delayed wash-out of mycolactone. An array of repurposed tuberculosis and leprosy drugs appears effective in vitro and in animal models. In clinical trials and observational studies, only rifamycins (notably, rifampicin), macrolides (notably, clarithromycin), aminoglycosides (notably, streptomycin) and fluoroquinolones (notably, moxifloxacin, and ciprofloxacin) have been tested.

Expert opinion: A combination of rifampicin and clarithromycin is highly effective but lesions still take a long time to heal. Novel drugs like telacebec have the potential to reduce treatment duration but this drug may remain unaffordable in low-resourced settings. Research should address ulcer treatment in general; essays to measure mycolactone over time hold promise to use as a readout for studies to compare drug treatment schedules for larger lesions of Buruli ulcer.

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Mycobacterium ulcerans; Buruli ulcer; treatment; pharmacology; clinical trials; pharmacokinetics

1. Introduction

1.1. Historical perspective

Mycobacterium ulcerans infection (Buruli ulcer) is a destructive infection of subcutaneous tissues resulting in ulcerative lesions of the skin, soft tissue, and sometimes bone [1–3]. The lesions are typically painless at initial presentation [4], although later, when lesions are ulcerated, patients may experience severe pain during wound care, especially, with dressing changes [5,6].

Buruli ulcer rarely kills [4,7], but it can certainly destroy people's lives; it is a disabling disease [8] associated with stigma, societal exclusion [9] and it has a large socio-economic impact, both for patients and for the health-care system [10]. Buruli ulcer has been listed by the World Health Organization among the 20 Neglected Tropical Diseases; it has been reported from over 30 countries; and it has a volatile, scattered epidemiology [11,12]. Severe and advanced disease is particularly common in scattered foci in Africa where most cases have been reported. Long delays in health care seeking is driven by socio-economic factors, beliefs, and attitudes prevailing in rural Africa [9,13–15]. The reservoir of the organism causing Buruli ulcer has not been fully elucidated, but the available evidence strongly suggests that it is environmental [16,17], like most non-tuberculous mycobacteria [18]. The mode of transmission is unclear, although it is believed to result from direct inoculation into the skin and subcutaneous fat [17,19]; human-to-human transmission is extremely rare [20]. For a long time, *M. ulcerans* infection was regarded as a condition that should be managed by surgery [4]. MacCallum et al. who first identified the causative organism from patients in Australia, reported surgical removal of the lesions [21].

The first description of Buruli ulcer on the African continent was by Albert Cook who worked as a missionary doctor in the end of the 19th century in the Mengo Hospital, near Kampala in Uganda [22]. The name was adopted after a report of multiple cases in the Buruli (now Nakasongola) district of Uganda [2,23].

1.2. First clinical drug trials

An early trial with clofazimine in the 1960 s was conducted by the British Medical Research Council, in the Buruli district in Uganda, near the Nile River [2]. The authors concluded that the drug did not have an appreciable beneficial effect [24]. A study in Côte d' Ivoire [25] failed to yield convincing evidence for a dominant role of antimicrobial treatment, partly, because of baseline differences in study groups, partly also because of limited follow-up of patients enrolled as participants in the study.

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Article highlights

- Buruli ulcer is a Neglected Tropical Disease caused by *Mycobacterium ulcerans*
- epidemiology is volatile; the micro-organism has an as yet poorly defined environmental reservoir; transmission is poorly understood, though direct inoculation in the subcutaneous tissues is likely
- disease features, including necrosis of subcutis and skin, immune down-regulation and lack of pain are all mediated by the secreted toxin, the polyketide mycolactone
- drug treatment appears highly effective, as evidenced by a recently reported clinical trial; resection surgery has become redundant and unnecessary
- oral drug treatment with 8-weeks rifampicin and clarithromycin appears safe and highly effective
- healing is slow, as a result of a slow wash-out of mycolactone that impairs spontaneous tissue repair
- research in wound care and early case finding in rural African settings are needed

1.3. From bench to bed – and back again

Meanwhile, several studies had demonstrated the in vitro susceptibility of *M. ulcerans* to an array of antimicrobial agents, both in vitro [26–32] as well as in experimental animal studies [31–35]. Eventually, the landmark study by Etuaful et al. [36] changed the thinking about the potential role of antimicrobial treatment for Buruli ulcer [37]. In that study, for the first time, the killing of *M. ulcerans* was demonstrated in early lesions of humans with culture – or PCR confirmed Buruli ulcer. Patients enrolled in that study were operated after pre-defined periods of antimicrobial treatment; in individuals treated for 4 weeks or more, no viable organisms could be cultured from these resected lesions. Based on this small study, subsequent studies employed 8 weeks duration of treatment, to keep a safety margin. The design of subsequent clinical trials to evaluate the role of antimicrobial treatment alone, without surgical resection of the lesions including a wide margin of apparently healthy tissue, changed, using complete healing without relapse at time point 52 weeks after the start of treatment, as the primary clinical end point or response parameter. Time to healing, subsequent functional limitations after healing, need for additional resection surgery, and adverse drug reactions, but not bacteriological end points were chosen as secondary end points.

2. Pathogenesis

2.1. The role of mycolactone

The pathology and pathogenesis of *M. ulcerans* infection have been described and reviewed [1,3,22,38,39]. The major virulence factor of *M. ulcerans* is a secreted polyketide exotoxin, mycolactone [40,41]. A secreted toxin had been earlier suspected [42] and demonstrated [43], but only after the chemical structure was elucidated [40], its dominant role in pathogenicity was gradually fully appreciated [44–46]. Different variants of mycolactone molecules occur [47]. Mycolactone A/B is the type occurring in Africa, and mycolactone C is present in Australia, although type A/B is more toxic than type C in vitro at similar concentrations, the clinical importance of these differences is not clear. The core and side chain of the mycolactone molecule are synthesized by three polyketide synthase enzymes encoded by a large plasmid – pMUM001 [48,49], while three additional cell wall-bound enzymes (MLSA1, MLSA2, and MLSB) are necessary to join the building blocks of the toxin [50]; these additional enzymes are produced by genes mup045, mup038, and mup053.

Mycolactone has three different important effects that impact on the pathogenesis of *M. ulcerans* infection – first, necrosis, and apoptosis of an array of host cells [51], including immune cells. Partly as a result of apoptotic pathways switched on in immune cells, and probably also, by a mechanism whereby mycolactone interacts with Sec61, a second effect, a down-regulation occurs in the overall immune defense [52,53]. Third, there is impairment of sensitivity (i.e., pain sensation) mediated by different mechanisms, including impaired nerve conduction of sensory nerves [54], through the interaction of mycolactone with AT2 R [55,56], as well as an impact on host Schwann cells, resulting in nerve damage [57–59].

Elucidating the dynamics of mycolactone [60] has changed the understanding of the pathogenesis of *M. ulcerans* infection. Animal models – especially, the mouse footpad model [31,32,53,61–63], have contributed substantially to our understanding of the pathogenesis and the response to antimicrobial treatment of *M. ulcerans* infection. In the mouse footpad model, swelling is correlated with the presence of mycolactone, even after the elimination of the bacterial load [46,60]. *M. ulcerans*, devoid of the plasmid pMUM001 despite being metabolically active, appears nonpathogenic [64,65]. For effective therapy, therefore, complete elimination of the *M. ulcerans* load may not be a prerequisite for clinical cure or effective treatment [66], at least not in the immuno-competent host [67]. Stopping the production of mycolactone in one way or other might suffice, while it clearly takes several weeks for mycolactone to be eliminated from host tissues infected with *M. ulcerans* [60].

2.2. Host immune suppression and immune reconstitution – paradoxical reaction

The gradual restoration of immune responses appears to mirror the gradual clearance of mycolactone from tissues and the bloodstream [51,68–70]. Several authors have described a transient increase in the inflammatory response following antimicrobial treatment of *M. ulcerans* infection [71–73]. This 'paradoxical' reaction that may occur in around 20% of cases typically occurs following antimicrobial treatment – the incidence peaks between 8 and 12 weeks following the start of treatment. The paradoxical reaction is believed to be associated with immune reconstitution after mycolactone has disappeared from the tissues. An association of paradoxical reactions with higher initial bacterial burden has been reported [73].

2.3. Secondary infection

Buruli ulcer lesions have necrotic sloughs at some point in time, during the course of the disease; secondary colonization by a large array of commensal bacterial populations including *Staphylococcus aureus* and *Pseudomonas*

aeruginosa is common [74–76]. It is currently unclear whether these organisms are colonizing 'innocent bystanders,' or whether these organisms cause additional harm. These secondary invaders might cause delayed wound healing or complications otherwise, particularly because some of these organisms harbor virulence factors associated with infectious complications [77]. Some of the secondary invading or colonizing organisms isolated from Buruli ulcer lesions are clustered, with evidence of nosocomial transmission [76,78]. In endemic areas in West Africa, an abundance of antimicrobial agents has been prescribed for suspected secondary infection. Based on the available evidence, much of this empirical treatment is irrational, and largely unjustified [79].

2.4. Measuring response to antimicrobial treatment

All of the above-mentioned considerations are important to fully understand the impact of pharmacological treatment directed at *M. ulcerans*. End points for in vitro studies are different from in vivo studies; and again, different in clinical studies. Clearly, antimicrobial treatment can only reduce the bacterial burden of *M. ulcerans*, and can stop the production of mycolactone; but for the lesion to heal, host immune and host repair mechanisms are critically important. Mycolactone molecules need first to be washed out from the tissues for these mechanisms to restore. If inflammatory responses increase following antimicrobial treatment, this may be erroneously taken for treatment failure [71]. This misinterpretation might in part explain why in some of the earlier trials with limited follow-up, treatment with clofazimine [24] or the combination of rifampicin and dapsone [25] seemed to fail.

2.5. Multi-drug treatment – rationale

In the early days of tuberculosis treatment, single-drug treatment was shown to result in a relapse of disease by drug-resistant organisms [80]. Multi-drug treatment regimens have since been used for mycobacterial infections, especially for tuberculosis [81], leprosy [82], but also for the non-tuberculous mycobacterial (NTM) infections [83], e.g., *M. kansasii* [84,85] and *M. avium-intracellulare* complex [86,87]. With high bacterial load, resistant mutations that occur by chance during cell division may result in the repopulation of lesions by drug-resistant mutants following monotherapy. Monotherapy results in failure and/or relapse with mono-resistant organisms, a phenomenon that has been recognized both in tuberculosis [88] and in leprosy [89]. In *M. tuberculosis* and probably also in other mycobacteria, drug resistance is not acquired by horizontal gene transfer, e.g., by inserting genetic mobile elements such as plasmids from other microbial species [90]. Besides a highly active core antimicrobial agent, a second companion drug should therefore always be in place to prevent treatment failure and relapse; this principle has also been applied in the pharmacological treatment of Buruli ulcer.

3. Pharmacotherapy for *M. ulcerans*: In vitro, in vivo and molecular susceptibility studies

Most in vitro studies to test susceptibility to antimicrobial agents have used egg-enriched media like Löwenstein-Jensen or Middlebrook 7H10 (7H10), as applied for other mycobacterial species, such as *M. tuberculosis*. Growth of *M. ulcerans* is relatively slow, with a replication time in liquid Middlebrook 7H12B-medium of 3–5 days [91]. Culture of *M. ulcerans* from clinical specimens has a limited yield, if compared with PCR-based diagnosis [92] but culture and sensitivity testing of cultured isolates is a robust test system. For experiments to test antimicrobial activity for agents to *M. ulcerans* in vitro, not only specific culture media but also temperature set at around 30°C is critical [21,91].

As explained above, *M. tuberculosis* and *M. leprae* have a human reservoir, and antimicrobial pressure resulting from the treatment of humans is the major driver of drug resistance. For *M. ulcerans* infection with no appreciable antimicrobial pressure on the reservoir of the organism, acquired drug resistance may be relevant for an individual, but acquired drug resistance has not been reported [93,94] and is not considered as clinically important. No specific drugs have been developed for *M. ulcerans* infection; all drugs currently in use and those tested are typically repurposed, most being specifically developed for tuberculosis or leprosy.

In vitro studies have reported on mycobacterial growth inhibition, with minimal inhibition concentrations using absolute concentration or dilution steps [26–30,33,95,96] and time-killing curves [97] assuming that such drug concentrations can be attained in the bloodstream of patients – and subsequently and presumably, at the site of their infection.

As mentioned earlier, a typical treatment schedule in use for mycobacterial infection including *M. ulcerans* infection would contain more than one drug. In vitro tests allow for multiple drug testing, using the so-called checkerboard analysis [62].

A review [98] summarized the in vitro data; several different classes of antimicrobials including macrolides, rifamycins, aminoglycosides, fluoroquinolones, as well as an array of new classes of drugs appear to have potential to kill, or inhibit growth, of *M. ulcerans*. An assay that assesses the potential of agents to arrest mycolactone production alone, without inhibiting growth or killing *M. ulcerans*, has been profoundly challenging [99].

In vitro culture systems testing antimicrobial efficacy using solid or liquid media have a steady concentration of a particular antimicrobial agent under study over time, with a gradual decay, depending on the chemical properties of the compound under study. An intrinsic weakness of such systems is that they poorly reflect antimicrobial concentrations fluctuating overtime during antimicrobial treatment as occurs in the bloodstream, as well as (conceivably) at the site of infection, in humans suffering from *M. ulcerans* infection. A system more closely resembling the real-life situation would be a hollow fiber infection model as used in tuberculosis drug research [100,101]. Such models not only mimic changing drug concentrations over time, but also compensate for possible chemical decay of pharmacological agents over time [102,103], which is particularly relevant for pathogens like mycobacteria with typically slow replication times.

In leprosy and tuberculosis, analysis of genetic mutations in regions of the genome coding for the molecular targets of antimicrobial agents have become increasingly important [90]. Mutations in the *rpoB* gene strongly correlate with (the level of) resistance to rifampicin [104,105], the first-line drug for drug-susceptible tuberculosis; rifampicin is also a core-drug for the antimicrobial treatment of leprosy. With whole-genome sequencing, multiple drug target mutations can be assayed predicting in vitro drug resistance [106].

In vitro, *M. ulcerans* is susceptible to clofazimine in most [107–110] but not all studies [95]. Some of the new tuberculosis drugs – bedaquiline [111], pretomanid, and linezolid have also been tested in vitro [33]. Telacebec (Q203) is a highly potent novel drug interfering with the respiratory chain of *M. ulcerans*; the inhibitory concentration dilutions of Q203, three times below the MIC, i.e., 15 or 7.5 ng/mL, did permit the growth of *M. ulcerans* strains; at 3.25 or 1.6 ng/mL, Q203 did not inhibit growth [96]. This new compound holds promise to reduce the duration of treatment [112] but no clinical studies to test this drug in Buruli ulcer have been started to date. TB47 is a novel compound developed for tuberculosis that appears to have a very low inhibition concentration for *M. ulcerans* as well [97].

As explained above, several animal models have been proposed, including the pig [113], and guinea pig [114], but the mouse footpad model has been the most widely used in vivo model to study antimicrobial treatment. This model was first developed by Fenner to study *M. ulcerans* infection [115]; Shepard [116] adopted it to test drugs for leprosy [31,62,117]. A Cochrane review provided a detailed summary of the evidence of pharmacological treatment of *M. ulcerans* infection [118].

Here, we discuss the most relevant antimicrobial agents tested in clinical trials; first, we summarize the evidence and considerations for each individual drug or drug class.

3.1. Rifamycins: rifampicin

Of the rifamycins, rifampicin [27,62,95] with MIC around 0.5 µg/ml is now considered a core drug in current treatment regimens. Rifapentine [63,111] has a longer half-life that might provide an advantage for patients in remote areas where intermittent therapy could be an asset. No clinical trials have evaluated regimens with rifapentine yet; the downside could be that with a companion drug with a much shorter half-life, inadvertent monotherapy would eventually result in drug resistance [95,119]. Rifampicin (just like the other rifamycins) interacts with the beta subunit of the bacterial ribosomal polymerase, encoded by the *rpoB* gene; if no mutations are present, rifampicin blocks synthesis of bacterial proteins. Mutations result in some fitness loss but compensatory mutations compensate for this fitness loss [120]. Rifampicin is generally well tolerated; liver damage, renal damage, a flu-like syndrome and skin eruptions are uncommon. The drug is rapidly absorbed from the intestine, with high (>90%) bioavailability; it is eliminated by the cytochrome p450 (notably, CYP3A4) enzyme system in the liver [121]. Over the course of the first weeks of treatment, this enzyme system is induced whereby the drug accelerates its own elimination, called auto-induction; this plateaus at around 3 weeks after the start of treatment [122]. With increased dosing (i.e., >10 mg/kg), bioavailability increases non-linearly; at 40 mg/kg, exposure increases ten-fold compared to dosing at 10 mg/kg [122]. Rifampicin interacts with several other drugs relevant for the treatment of *M. ulcerans* infection. The therapeutic window is relatively large; standard dosing tested in *M. ulcerans* infection was derived from treatment schedules in use in tuberculosis and leprosy, set at 10 mg/kg bodyweight; higher doses up to 35 mg/kg have been tested in patients with tuberculosis without increased toxicity [123,124]. In the mouse footpad model, high dose rifampicin resulted in rapid sterilizing activity – faster than at standard doses – potentially allowing for shorter treatment duration [125]. CYP3A4 induction results in enhanced clearance of macrolides, including clarithromycin and azithromycin; and some of the fluoroquinolones, notably moxifloxacin.

3.2. Aminoglycosides: streptomycin

Several aminoglycosides including amikacin [33,126] and kanamycin [127] have been tested in *M. ulcerans* mouse models. The aminoglycoside streptomycin was initially chosen as the companion drug of rifampicin in the first proof-of-principle study by Etuaful et al. to evaluate the potential of antimicrobial agents possibly replacing surgery as the primary mode of treatment [36]. Most antimicrobial agents that interfere with protein synthesis are bacteriostatic, although aminoglycosides interfere with protein synthesis by binding to the 30 S subunit of bacterial ribosomes, they are bactericidal drugs. Their efficacy increases with increasing peak plasma concentration [128]. Used as an intramuscular injection, children, and adults alike suffer pain, if this treatment is continued for a full duration of 8 weeks; the dose was chosen at 15 mg/kg body weight, based on experience in tuberculosis [129,130], and this worked well in the animal model [33,117]. Streptomycin being an aminoglycoside has appreciable renal and acoustic toxicity [131] and it is not considered safe during pregnancy. Subsequent trials in humans have therefore tried to either reduce the number of streptomycin injections by switching after 4 weeks of streptomycin-rifampicin treatment to an oral schedule without injected streptomycin [132], or to only 2 weeks with injected streptomycin and then, switched to the oral treatment [133]. Four weeks of streptomycin were non-inferior to the full 8 weeks of streptomycin injections [132], while without a randomized comparison, 2-weeks streptomycin treatment had a high success rate [133]. An open-label randomized study compared fully oral therapy with 8 weeks of standard streptomycin-rifampicin (Clinicaltrials.gov: NCT01659437) [134]; the final report was recently submitted for publication. Of the 151 patients treated with rifampicin and streptomycin, 144 patients had healed lesions without relapse at the pre-defined time point 52 weeks after the start of treatment – 95.4 (IQR: 90.7–98.1)%, while 140/146 patients on rifampicin/clarithromycin treatment – 95.9 (IQR: 91.3–98.5)% were healed, showing non-inferiority. Median time to healing was 24 (IQR, 8–28) weeks in the streptomycin/rifampicin treated patients, and 16 (IQR, 8–25) weeks in the clarithromycin/rifampicin treated patients. Significantly more patients on streptomycin treatment had ototoxicity. In conclusion, we expect that streptomycin

will no longer be maintained among the recommended modes of treatment for *M. ulcerans* infection.

3.3. Macrolides: clarithromycin

Macrolides have excellent activity against *M. ulcerans*, both in vitro [29] as well as in vivo [31,34,35,111]. They disturb bacterial protein synthesis, and are bacteriostatic. They act by inhibiting peptidyltransferase, while binding to the 50 S subunit of the bacterial ribosome – resistance is mediated by mutations in the A2058 nucleotide of the 23 S rDNA [135]. Most studies were conducted with clarithromycin although in vitro testing suggests that azithromycin is at least as effective [31]. Bioavailability of the newer macrolides (clarithromycin, azithromycin) is around 50%; drug penetration in tissues is excellent while it accumulates in some cells like granulocytes. Drug elimination of clarithromycin is by 14-hydroxylation in the liver, by the CYP3A4 enzyme system. The drug and its 14-OH metabolite tend to accumulate with renal clearance below 30 ml/h [136]. Unfortunately, the 14-OH metabolite was not active for five strains of *M. ulcerans* tested [137]. The largest clinical drug trial for Buruli ulcer, sponsored by the WHO [134], established that the combination of clarithromycin and rifampicin was to be preferred to the earlier recommended combination of streptomycin and rifampicin, considering that its efficacy was non-inferior, with a success rate around 95%, and associated with significantly less adverse drug effects. Although the clinical response was highly favorable in PCR-confirmed lesions ≤10 cm cross sectional diameter, drug–drug interactions are a concern; clarithromycin reduced rifampicin elimination which is perhaps a benefit rather than a concern [137], but clarithromycin elimination was enhanced by CYP3A4 enzyme induction, which would call for slightly higher dosage than 7.5 mg/kg as tested; in the WHO trial, clarithromycin was therefore administered as extended release formulation at 15 mg/kg but it is unclear whether this would offer any benefit compared to immediate-release medication dosed at 7.5 mg/kg.

3.4. Fluoroquinolones: moxifloxacin, ciprofloxacin

Fluoroquinolones have been shown to be bactericidal in vitro and in vivo [28,30,31,33–35,111]. Safety concerns with fluoroquinolones in childhood and in pregnancy have restricted their use, particularly in Africa where children are predominantly affected. In Australia where the majority of patients are elderly, the drugs have been widely used [138–140] with an excellent safety profile [141]. Fluoroquinolones act by interfering with bacterial DNA [142]; in tuberculosis, the promise of shorter duration on therapy has not been fulfilled, perhaps because of sub-optimal drug exposure [143]. Fluoroquinolones, and especially moxifloxacin, have potential for QTc prolongation, but the clinical impact (i.e., potentially fatal cardiac arrhythmia – known as Torsade de Pointes) is not always obvious; the number of reported fatal events has been low, and no cases have been reported to date in the context of treatment for Buruli ulcer. Drug–drug interactions with rifampicin that induces CYP3A4 pathways and thereby enhance drug elimination, are a conceptual disadvantage [128].

3.5. Cotrimoxazole, dapsone

Cotrimoxazole has only recently been studied for possible use in tuberculosis [144]. Around 50% of *M. tuberculosis* strains tested appeared susceptible to cotrimoxazole [145]. *M. ulcerans* was tested susceptible in one publication with a small number of *M. ulcerans* isolates, that was published in French [146]; one small clinical study claimed a beneficial effect in patients but the study had limited methodological strength [147]. *M. ulcerans* strains when tested in vitro for susceptibility to dapsone, an anti-leprosy drug, and assessed as susceptible in vitro [26,148]. One clinical trial evaluated dapsone in combination with rifampicin; due to baseline differences in study arms, and limited follow-up, the results were basically inconclusive [25].

3.6. Miscellaneous drugs: clofazimine, bedaquiline, linezolid, telacebec, TB47; beta-lactams

As mentioned above, there are no reports to date on the clinical use of these agents in patients, except for the trial on clofazimine monotherapy in Uganda in the 1970 s [24]. In animal models, it has potential for shortening therapy [95,109,110] when used in combination regimens, although one report claims that the drug is not very active in vitro [95]. Studies report a high volume of distribution due to its lipophilic chemistry; and its associated sterilizing capacity [109].

Clofazimine has the disadvantage of discoloration of skin, which restricts its prolonged use in Asians that dislike this potentially stigmatizing side effect; it has been used extensively in multi-drug resistant tuberculosis with excellent results [149].

Bedaquiline might be an asset but clearly, the price might be prohibitive, while the antimicrobial spectrum and pharmacokinetics might be close to clofazimine. Linezolid is generally considered too toxic for a condition that it not lethal, like multi-drug resistant tuberculosis [150,151]. Telacebec (Q203) [96,112] and TB47 [97] deserve further clinical testing, because of their potential to shorten treatment duration.

Beta-lactam antimicrobial agents – especially, carbapenems have attracted attention for the treatment of MDRTB, and have also been studied in vitro for their effect on *M. ulcerans*. In the assays used, inhibitory concentrations were unachievable when used alone, but in a checkerboard analysis, a strong synergistic effect was noticed, especially when used in combinations with three active drugs, with or without the beta-lactamase inhibitor clavulanic acid [152]. These drugs are conceptually attractive because of the generally low toxicity and safety in pregnancy; as their action is time-dependent, prolonged exposure would be needed; and most agents tested were only available as parenteral formulation, which would hamper their applicability in clinical practice.

4. Clinical considerations and recommendations

With the evidence provided by the largest clinical trial to date, we believe that a fully oral regimen of rifampicin – at least 10 mg/kg, but perhaps a bit more – and clarithromycin – either in an extended-release (15 mg/kg daily) or as immediate

release, 7.5 mg/kg, or slightly more – would be an excellent choice, both in children and in adults. The safety profile is excellent; and efficacy is high if lesions are limited. No well-designed studies have addressed the question whether 8 weeks should be considered standard, and whether treatment duration could be individualized. Unfortunately, no biomarkers have been developed or validated to help guide individual decisions on treatment duration. Observational studies suggest that in some cases, less than 8 weeks could suffice [153,154]. In Australia, extensive clinical expertise – albeit without formal comparative clinical studies – supports the use of fluoroquinolones [139,140,155] with a highly acceptable safety profile [141].

After the introduction of antimicrobial treatment as a first-line treatment modality, the role of surgery has become limited. With surgery alone, treatment failure and relapse occurred in 18 [156] to as high as 47% [157]. Extensive resection with a margin of apparently healthy tissue to prevent relapse is no longer indicated, as under antimicrobial treatment, relapses have virtually gone extinct [132,133,158,159]. Postponing the decision about surgery from the time just after completion of antimicrobial therapy to 14 weeks after the start of treatment did not result in delayed healing, relapse, or any other adverse effect for patients. A randomized study evaluating postponement of decisions about surgery showed only beneficial effects for patients for whom surgery decisions were postponed. There was even a reduction in the number of patients operated on; indeed, in significantly more patients in whom decisions were postponed, surgery was deemed redundant without any ill effect [160].

5. Expert opinion – future perspectives

Antimicrobial treatment has brought many advantages for patients with Buruli ulcer, but some questions have remained unanswered. Clinical studies can only address relatively simple questions; and although randomized trials provide the highest level of evidence to guide therapy, in clinical practice, many decisions require individualized decisions. For some infections, like community-acquired pneumonia, duration of antimicrobial treatment can be safely individualized and indeed stopped after fulfilling criteria to achieve clinical stability [161]. For many infections, like tuberculosis, no robust biomarker or decision rule have been developed that can be used to individualize treatment duration. For tuberculosis, notably for patients with drug-resistant forms of tuberculosis, individualized treatment has primarily focused on the selection of drugs. The concept of tailoring treatment according to pharmacokinetic/pharmacodynamic (PK) modeling combined with susceptibility testing [90,162,163], using drug susceptibility essays for each individual drug in the treatment schedule, combined with adjusting dosing based on drug exposure measurements, i.e., therapeutic drug monitoring [164] holds promise for tuberculosis, but may not necessarily be the way forward for Buruli ulcer individualized treatment. One problem with this approach is, that phenotypic in vitro drug susceptibility testing using solid or liquid culture media with steady single drug concentrations below the breakpoint hardly reflects what happens in infectious foci in patients harboring the pathogen under study; the hollow fiber infection model mimics these variable drug concentrations in the bloodstream over time with continued nutritional, pCO_2, and PO_2 conditions as happens in the bloodstream of patients [100]. Even modeling drug concentrations in the bloodstream of patients may still differ from what happens at the site of infection – and at least in tuberculosis, some of these assumptions prove wrong [165]. Indeed, typically, blood drug concentrations in patients vary following ingestion, with resorption, distribution, and elimination following a curve of rising and falling concentrations over time. This is especially important for microorganisms with slow replication like mycobacteria, where drug concentrations tend to fall over time due to chemical instability [103,166]. Despite these considerations, telacebec, for example, showed an impressive activity in the mouse footpad model, confirming the in vitro data [91,107]. Use of auto – or bioluminescent strains of *M. ulcerans* may have the potential to reduce and refine animal experimentation [127].

In vitro susceptibility testing does not take host immune defenses into consideration.

We believe that it would be unlikely that patients with adequate immunity, small lesions, and adequate nutritional status would require the same treatment, with the same treatment duration, as patients with impaired immunity, large lesions, impaired nutritional status, and poor general health. Individualized treatment duration seems, therefore, a logical next step, and observational data indeed suggest that some patients do well after less than even 6 weeks of antimicrobial treatment [153,154]. It would, however, be extremely challenging to design studies to address questions about individualized treatment duration. Future studies on optimal duration and composition of treatment in patients with lesions larger than 10 cm cross-sectional diameter should perhaps require a microbiological, not a clinical end point. Wound healing, a stable scar, or full epithelialization at 12 months after the start of antimicrobial treatment in larger lesions is probably not the best way to compare, or assess, antimicrobial treatment modalities. As culture is not very sensitive, and perhaps less relevant than assessing a stop of mycolactone production, a logical way to assess the efficacy of antimicrobial treatment would be, to measure mycolactone in lesions over time [167].

As explained above, wound healing is the result of a combination of appropriate antimicrobial treatment, combined with principles of optimized wound care. Wound care is insufficient if the patient has poor nutrition, hyperglycemia, or anemia; these factors should be addressed first. Local wound care includes removal of infected necrotic slough; regular cleaning of the wound surface; applying a non-adherent (e.g., Vaseline-based) wound surface cover so as to avoid damage of the delicate host microvasculature, and integrity of host epithelial and fibroblast cells, with optimized humidity of the wound surface. If a wound is still purulent or discharging, dressing materials should have the adequate absorptive capacity, and compression, combined with optimized mobilization to stimulate arterial vasculature, and optimized venous and lymphatic return. Vascularization may occasionally require plastic surgical intervention. Not all of these supportive factors require specific clinical studies. Still, we believe that the body of evidence for optimal wound care is limited, and some controversial issues like questions about

type and methodology of compression therapy would greatly benefit from answers provided by formal randomized comparisons.

Simple questions, e.g., the optimal number of dressing changes per week, might also be addressed in formal studies; some of these questions might be answered by enrolling a variety of different wounds (e.g., Buruli ulcer, tropical ulcer, venous and diabetic ulcers), as much of current wound care science is expert opinion-based, without a strong scientific evidence base. In summary, we believe that wound management [168,169] would be an important area of future research to improve outcomes for patients with Buruli ulcer. The role of debridement surgery, extent of removal of the necrotic slough, or timing or type of skin grafting has also been little studied [160], there is a striking variability in surgical practice that is not explained by differences in patient populations, or clinical presentations of wounds, but rather by individual doctors caring for these patients [170]. As resection surgery does not generally bring a clear benefit to patients, this practice should best be discouraged, especially in poor-resourced settings where surgery is much more of a concern than in affluent settings where specialist care is widely available – but even there, the benefit of resection surgery is probably over-rated.

Lesions at critical sites like the face or the genital organs deserve special attention [171]. Finally, prevention of disabilities [8,172] and early case finding [173] as well as stigma reduction deserves as much attention as further development of shorter and more effective antimicrobial therapy. Clearly, the currently available evidence that oral antimicrobial treatment is the best treatment to date, is good news for the young patients in poor-resourced settings in West Africa.

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